ANTIHYPERGLYCEMIC ACTIVITY OF ALCOHOL AND AQUEOUS EXTRACTS OF *PANDANUS FASCICULARIS* LAM. ROOTS IN ALLOXAN INDUCED DIABETIC RATS

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Running title - Antihyperglycemic Activity of *P. fascicularis*.

Summary

Pandanus fascicularis Lam. (Pandanaceae) is known as *Ketaka* in Ayurveda. The antihyperglycemic activity of alcohol and aqueous root extracts of *Pandanus fascicularis* Lam, was evaluated in alloxan induced diabetic rats. Preliminary phytochemical and acute toxicity studies of root extracts were carried out. The antihyperglycemic activity of extracts was compared with reference drug glibenclamide. Extracts were given orally doses of 500 and 750 mg/kg body weight. Blood samples were collected on 0^{th}, 7^{th}, 14^{th} and 21^{st} days by orbital sinus puncture under mild ether anesthesia, for serum glucose estimation. On 21^{st} day glycosylated hemoglobin was estimated along with glucose. Extracts were found to produce lowering of blood glucose level in alloxan induced diabetic rats, when compared to diabetic control. Glycosylated hemoglobin level significantly reduced when compared to control (p value < 0.001). Alcohol and aqueous extracts at both doses (500 and 750 mg/kg body weight) restored serum glucose level near to normal. The results of this study substantiate the traditional use of this drug in the treatment of diabetes.

Keywords: Alloxan, antidiabetic activity, glibenclamide, glycosylated hemoglobin, *Pandanus fascicularis.*

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Introduction

Diabetes mellitus is increasing alarmingly world wide and inviting major health problems from its complications[1]. Diabetes mellitus is a group of disorders characterized by interruption in carbohydrates, protein and fat metabolism due to complete or relative deficiency of insulin secretion and/or insulin action[2]. The increasing incidence of the disease worldwide may be due to sedentary life style, unhealthy diet, obesity and other predisposing risk factors[3]. Ethnobotanical knowledge played a particularly significant role in historical diabetes therapies, with over 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetic indications[4]. The evaluation of these plants and of their active natural principles is a logic way of searching for new drugs to treat this disease[5].

The Ayurvedic drug *Ketaka* is *Pandanus fascicularis* Lam. belonging to the family Pandanaceae[6,7]. More than 500 species are known to occur in genus *Pandanus*, out of these 36 species have been recorded in India[8]. It is widely distributed in India over coastal districts of Orissa (especially in Ganjam), Andhra Pradesh, Tamil Nadu, and to some extent in parts of Uttar Pradesh[9]. Anti-oxidant activity of constituents isolated from *P. odoratissimus* has been reported[10]. The crude alcohol extract of *P. tectorius* has diuretic activity[11]. In Ayurveda *Ketaka* used for rheumatism, headache, anorexia, indigestion, constipation, eye diseases, leprosy, hypoglycemic activity[12,13].

Hence, the present work was undertaken to establish the hypoglycemic activity in alcohol and aqueous extracts of root of *P. fascicularis*. in alloxan induced diabetic rats and to compare it with diabetic control group.

Material and Methods

Plant material

Fresh adventitious roots of *P. fascicularis* Lam. were collected from the vicinity of Tirunelveli, Tamil Nadu during March 2007. The plant was identified and authenticated by Dr. S. N. Yoganarasimhan (Taxonomist and Research Co-ordinator, M. S. Ramaiah college of Pharmacy, Bangalore). A voucher herbarium specimen (Jagdish chandra nagar 021) of the plant species has been deposited in the herbarium of Department of Pharmacognosy of M. S. Ramaiah College of Pharmacy, Bangalore, India for future reference. The collected roots were washed thoroughly in tap water to remove any unwanted matter and then dried under shade. The dried roots were powdered and stored in an airtight container. A voucher sample of tested material for experiment studies has been preserved at the crude drug museum of M. S. Ramaiah college of Pharmacy.

Preliminary phytochemical study

Preliminary phytochemical screening of alcohol and aqueous extracts were carried out[14].

Preparation of alcohol root extract

Coarsely powdered, shade dried roots of *P. fascicularis* were charged into a soxhlet apparatus and successive hot extraction was carried out using ethanol (70% v/v) for 24 h. The liquid extract was concentrated in rotary flash evaporator at a temperature not exceeding 50ºC (yield 10.62% w/w). The alcohol extract was formulated as a suspension in distilled water using 2% v/v Tween-80 as suspending agent for animal studies.

Preparation of aqueous root extract

Coarsely powdered, shade dried roots of *P. fascicularis* was macerated with chloroform water for 24 h. The macerate was filtered and filtrate concentrated in rotary flash evaporator (yield 12.80% w/w). Aqueous extract was prepared by dissolving in distilled water for animal studies. The extracts were preserved in a desiccator for further experiments.

Preparation of standard drug solution

Glibenclamide (Bal Pharma, Bangalore, India) was used as the reference drug for evaluating antihyperglycemic activity. Glibenclamide suspension was prepared in distilled water using Tween-80 as suspending agent.

Animals

Swiss albino mice (20-30g) and Wistar rats (170±10g) of either sex were used for the study. The animals were procured and housed in the animal house of M. S. Ramaiah College of Pharmacy Bangalore, 2 weeks prior to the experiment, for acclimatization. Animal house was well maintained under standard hygienic conditions, at a temperature (25±1°C), room humidity (60%±10%) with 12 h day and night cycle with pellet diet (Sai Durga Feeds and foods, Bangalore) and water *ad libitum*. Paddy husk was provided as bedding material and was changed everyday. All the pharmacological experiments were carried out as per CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) norms after obtaining approval of the Institutional Animal Ethics Committee of M. S. Ramaiah College of Pharmacy, Bangalore.

Acute toxicity studies

These studies were carried out to study acute toxic effects and determine minimum lethal dose of drug extracts. Swiss albino mice of either sex fasted overnight, were used for the study. Each extract was orally administered at doses of 30, 100, 300, 1000 and 3000 mg/kg to separate groups of mice. Subsequent to administration of drug extracts, the mice were observed closely for the first 3 hours, for any toxic manifestations, such as increased motor activity, salivation, clonic convulsions and death, followed by observations at regular intervals for 24 hours. The observations were continued for further 7 days[15].

Induction of diabetes mellitus

Diabetes mellitus was induced in Wistar rats by single intraperitoneal injection of 120mg/kg body weight of alloxan monohydrate (Spectrochem Pvt. Ltd., Mumbai, India), dissolved in normal saline[16]. After 5 days blood samples were collected by retro-orbital puncture under mild anesthesia and serum glucose levels were monitored. Rats showing serum glucose level above 200 mg/dl were used for the antihyperglycemic evaluations and randomly divided into groups 2 to 7.

Experimental design

The animals were divided into 7 groups of 6 rats each[17].

Group 1 - Normal healthy control
Group 2 - Untreated alloxan diabetic rats
Group 3 - Alloxan diabetic rats were treated with glibenclamide (500µg/kg, p.o.)
Group 4 - Alloxan diabetic rats were treated with aqueous extract (500 mg/kg p.o.)
Group 5 - Alloxan diabetic rats were treated with aqueous extract (750 mg/kg p.o.)
Group 6 - Alloxan diabetic rats were treated with alcohol extract (500 mg/kg p.o.)
Group 7 - Alloxan diabetic rats were treated with alcohol extract (750 mg/kg p.o.)

The treatment schedule was once daily for 21 days and animals were fed on laboratory diet of pellet chow and water *ad libitum*. They were fasted for 18 h prior to blood withdrawl. Blood samples were collected on 0^{th}, 7^{th}, 14^{th} and 21^{st} day from the start of treatment, by orbital sinus puncture under mild ether anesthesia. Serum was separated by centrifuging blood at 6000rpm for 15 mins. Serum glucose estimation was performed by end point method, using semi auto-analyzer (B4B chemistry analyzer CA-2005) with the help of Liquid gold diagnostic kit (Span Diagnostic Limited, Surat, India). On 21^{st} day estimation of glycosylated hemoglobin was also performed using UV- visible spectrophotometer (Schimadzu 1601) with the help of Glycohemoglobin reagent kit (Coral Clinical System, Goa, India).

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test ($p < 0.001$) using instat software. All values were expressed as Mean ± SEM.

Results

Alcohol and aqueous extracts of *P. fascicularis* did not exhibit any toxic symptoms or mortality. The extracts were found to be safe at dose level of 3000 mg/kg, indicating the high margin of safety of the drug.

The studies on the alcohol and aqueous extracts of *P. fascicularis* revealed that the extracts caused significant reduction in serum glucose levels in hyperglycemic rats at both dose levels (500 and 750 mg/kg body weight), when compared to diabetic control. The reduction in mean serum glucose level was dose dependent. The serum glucose levels of normal and alloxanized rats on 7^{th}, 14^{th} and 21^{st} days are depicted in Table-1.

Table 1. Effect of alcohol and aqueous extracts of roots of *P. fascicularis* Lam. on blood glucose level in diabetic rats

Sl. No.	Group (Treatment)	Blood glucose level (mg/dl)			
		0^{th} day	7^{th} day	14^{th} day	21^{st} day
1.	Normal untreated	126.31±10.40	123.26±09.55	110.61±09.27	114.10±08.43
2.	Diabetic untreated	388.12±04.66	447.17±54.21	416.98±35.58	368.64±17.38
3.	Diabetic rats treated with glibenclamide (500µg/kg)	407.41±20.70	235.97±27.35***	179.85±27.56***	123.68±12.31***
4.	Diabetic rats treated with aqueous extract (500mg/kg)	393.39±46.86	292.71±28.72**	180.50±30.49***	148.13±14.10***
5.	Diabetic rats treated with aqueous extract (750mg/kg)	397.72±23.60	231.23±17.52 ***	166.39±04.64***	120.98±17.11***
6.	Diabetic rats treated with alcohol extract (500mg/kg)	391.34±13.96	281.58±23.03**	234.46±26.81***	168.66±12.55***
7.	Diabetic rats treated with alcohol extract (750mg/kg)	384.42±14.97	274.05±15.40***	174.85±8.86***	124.99±7.71***

Values expressed as Mean ± SEM.
One Way ANOVA (p value < 0.0001)
Tukey Kramer Multiple Comparison Test (*** p < 0.001, ** p < 0.01) in comparison with diabetic control group.

Glycosylated hemoglobin was significantly reduced in groups treated with extracts, when compared to control. The glycosylated hemoglobin levels on 21st day are depicted in Table-2.

Table 2. Effect of and aqueous extracts of roots of *P. fascicularis* Lam. on glycosylated hemoglobin levels on 21st day

Sl. No.	Group (Treatment)	Glycosylated hemoglobin (%) on 21st day
1.	Normal untreated	3.90±0.11***
2.	Diabetic untreated	12.28±0.12***
3.	Diabetic rats treated with glibenclamide 500µg/kg	4.19±0.21***
4.	Diabetic rats treated with aqueous extract 500mg/kg	5.43±0.55***
5.	Diabetic rats treated with aqueous extract 750mg/kg	4.31±0.19***
6.	Diabetic rats treated with alcohol extract 500mg/kg	5.91±0.38***
7.	Diabetic rats treated with alcohol extract 750mg/kg	4.66±0.14***

Values expressed as Mean ± SEM.

One Way ANOVA (p value < 0.0001)

Tukey Kramer Multiple Comparison Test (*** $p < 0.001$) in comparison with diabetic control group.

Discussion

Oral antihyperglycemic agents and insulin are widely used in diabetes treatment, but they also have prominent side effects and fail to alter the course of diabetic complications. Effective control of the blood glucose level is a key step to prevent or reverse diabetes complications[18]. Alloxan is a potent diabetogen. The toxic action of alloxan on pancreatic β-cells is the sum of several processes such as oxidation of essential -SH groups, inhibition of glucokinase, generation of free radicals and disturbances in intracellular calcium homeostasis[19]. The extracts exhibited hypoglycemic effects comparable with the antidiabetic drug glibenclamide. Glibenclamide causes hypoglycemia by increasing insulin secretion from pancreas and by reducing hepatic clearance of the hormone[20].

This study shows that both aqueous and alcohol extracts exert significant dose dependent reduction in serum glucose levels in diabetic rats, from day 7 onwards. The observed antihyperglycemic effect of *P. fascicularis* may be due to the increase in pancreatic insulin release or the outcome of some extrapancreatic effects of the drug, which requires further investigation.

Preliminary phytochemical screening revealed presence of phenolic compound, tannins and flavonoids in aqueous and alcohol extracts. As per literature, constituents such as phenolic compound, tannins and flavonoids are known to reduce glucose level in diabetic condition[21]. Hence, the potent antihyperglycemic activity of *P. fascicularis* root may be due to the presence of phenolic compounds, tannins, and flavonoids.

Conclusions

Many plants are mentioned in Ayurveda for hypoglycemic effects (Prameha). The results of this study substantiate the traditional use of this drug in the treatment of diabetes. The roots of *P. fascicularis* may be a potential source of drug for management of diabetes mellitus. Further detailed studies may be carried out to identify the active principle(s) responsible for the hypoglycemic effect and to understand the exact mechanism of action.

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